

CONSTRUCTION AND EXPRESSION OF QUARTET RECOMBINANT PEPTIDE SURFACTANT FOR EOR APPLICATION

KONSTRUKSI DAN EKSPRESI REKOMBINAN KUARTET SURFAKTAN PEPTIDA UNTUK APLIKASI EOR

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ABSTRAK

Kelemahan utama produk surfaktan peptida SUPEL yang telah dikembangkan untuk aplikasi EOR adalah kurang stabil pada suhu tinggi. Penelitian ini bertujuan untuk membuat prototipe konstruksi surfaktan peptida tersebut secara rekombinan dengan merangkai empat urutan SUPEL secara linear. Teknologi rekombinan kuartet dapat menghasilkan surfaktan peptida yang memiliki sifat reversible biosurfactant, yaitu aktif pada suhu tinggi dan non aktif pada suhu rendah. Multiple SUPEL Construction (MSC) yang dikembangkan dalam penelitian ini menggunakan sintetik DNA menghasilkan SUPEL dalam rangkaian empat untai yang dapat melipat di saat suhu normal dan membuka jika dipanaskan. Hasil analisis SDS PAGE menunjukkan konstruksi MSC dapat diekspresikan dengan cara induksi IPTG dan sel dipanen pada suhu 90°C. Penelitian ini membuktikan bahwa konstruksi dan ekspresi kuartet SUPEL telah berhasil dilakukan dengan menghasilkan peptida pada ukuran yang sesuai.

Kata Kunci: surfaktan peptida, rekombinan kuartet, SUPEL, stabilitas panas, EOR

ABSTRACT

The main drawback of the SUPEL peptide surfactant product which has been developed for EOR application is it is unstable at a high temperature. This research is aimed at generating the prototype of peptide surfactant construction in recombinant by stringing up 4 SUPEL linear sequences. Quartet recombinant technology can produce the peptide surfactant characterized as reversible biosurfactant, which is active at high temperature but inactive at low temperature. Multiple SUPEL Construction (MSC) that was developed in this research is using synthetic DNA and producing SUPEL in 4 sequences that can flip at normal temperature and can open when heated. SDS PAGE analysis results show that MSC construction can be expressed by inducing IPTG and cell harvested at 90°C. This research proves that construction and expression of the SUPEL quartet has been achieved by producing the peptide at an ideal size.

Keywords: peptide surfactant, quartet recombinant, SUPEL, thermal stability, EOR

I. INTRODUCTION

The usage of surfactant in chemical injection for enhanced oil recovery has been proved capable of increasing oil recovery from mature producing fields (Zhu et al. 2013). Surfactant has a function of lowering the interfacial tension (IFT) of water-oil

through adsorption by cutting the hydrogen chain at the molecule surface. The higher the level of causes the lower the IFT. For the success of surfactant injection, IFT must be lowered in range of 10 to 30 dyne/cm when water injection is typically less than 10⁻³ dyne/cm. The very low value of IFT will reduce

capillary pressure in rock pore so the oil displacement efficiency to production wells is increased. Surfactant can also be adsorbed at the solid-liquid phase border, so it changes the contact angle of solid-liquid. This situation is then changing rock wettability from oil-wet into water-wet so the trapped oil in reservoir pores can easily flow to production wells (Wang & Mobanty 2014).

The EOR surfactant that is mostly used in industry nowadays is made from petroleum sulfonates as a result of petrochemical synthetic (Gao & Mani 2012; Zhang et al. 2015). This surfactant system has one or more nonpolar tails as hydrophobic which is linked with the polar head as hydrophilic. The performance of this surfactant to enhance the microscopic displacement efficiency in a reservoir is heavily affected by the hydrocarbon structure (Barnes et al. 2012). Therefore, surfactant formulation is often a time consuming process and also high cost because the activity of the surfactant system must be tailored to the oil characteristics and reservoir conditions (Buijse et al. 2012).

One of the alternatives to overcome this drawback is by using a peptide biosurfactant. Peptide could be engineered to have an amphiphilic characteristic - the separation of hydrophilic and hydrophobic - by adjusting the composer of amino acids position. Therefore, peptide surfactant is also known as reversible surfactant, which is switchable to be either active or inactive under certain

conditions. This characteristic enables the surfactant system formulation for certain oil characteristics and reservoir conditions to be done more effectively. The other benefit of peptide biosurfactant is it is cheaper and eco-friendly compared to petroleum sulfonate based surfactant.

The peptide surfactant product called SUPEL (Peptide Surfactant LEMIGAS) has been tested on several oil samples with different reservoir conditions (Jaya et al. 2012; Usman 2015). The main drawback of developed SUPEL is it is unstable at high temperature. SUPEL structure can only stick out at room temperature and soon broke up when tested under reservoir conditions. In addition, the used SUPEL product is a chemically synthetic result, which is costly for large scale production.

This research is aimed at producing SUPEL using recombinant DNA technology. The SUPEL quartet design or multiple SUPEL construction (MSC) used in this research can produce active peptide surfactant at high temperature and is easily harvested. Reversible surfactant is constructed into pET 32b plasmid, then recombinant SUPEL expression is analyzed in plasma inside Escherichia coli BL21 (DE3) cell.

II. METHODOLOGY

A. Codon Usage SUPEL Optimization

In this research Codon Adaptation Index (CAI) with reference of gen that is high expressed in E. Coli K12. MCS is produced with pET 32b construction

Table 1
Codon usage optimization in E. Coli based on SUPEL amino acid sequence (Maloy et al. 1996)

Codon	Amino Acid	Appearance (%)	Codon	Amino Acid	Appearance (%)
ATG	Met (M)	100	CTG	Leu (L)	55
GAC	Asp (D)	41	GAA	Glue (G)	70
TTC	Phe (F)	49	AAC	Asn (N)	61
TCT	Ser (S)	19	ATC	Ile (I)	46
TCT	Ser (S)	19	CTG	Leu (L)	55
ATG	Met (M)	100	GAC	Asp (D)	41
GCT	Ala (A)	19	AAA	Lys (K)	76
AAA	Lys (K)	76	GCT	Ala (A)	19
CTG	Leu (L)	55	CGT	Arg (R)	42
TGC	Cys (C)	57	AAC	Asn (N)	61
CAC	His (H)	48	TCT	Ser (S)	19
ACC	Thr (T)	43	TAA	Sto (P)	62

that bring 4 thread (4x) of SUPEL acid. The sequence of SUPEL acid as a result of codon usage optimization which is used in this research is shown at Table 1 (Maloy et al. 1996).

B. MSC Design

Peptide surfactant sequence in this MCS construction comes from synthetic gen, GenART. Then, at this sequence the suitable restriction is added in order to be able to put in the pET 32b expression vector, that is BamHI and HindIII. SUPEL 4 threads sequence are purified from the cloning vector by cutting that cloning vector using BamHL and HindHL.

Expression Vektor pET 32b is also cut using the same enzyme so SUPEL 4 threads sequence fragment can be ligated into pET 32b expression vector using T4 DNA ligation. Recombinant plasmid confirmation is done using a restriction enzyme and the PCR method.

C. MSC Expression Test

MSC single construction colony which is inside BL21 strain E. Coli cell (DE3) is cultured in M9 plus ampicilin for 16 – 18 hours. One millimeter of that culture is moved into 100 mL M9 plus ampicilin 100 mg/L and agitated at 180 rpm at 37°C for 4 hours until OD_{600} reach 0,45 and then induced with IPTG 0,1 mM. The peptide harvest process is done in two ways: by cracking cell and non-cracking cell. Bacterial cell which has been harvested is then pelletized. The unheated cell is then resuspended

with soluted in millique water. Cracking cell process is done with several optimizations, that is by heating up to 90°C, and 90°C with the addition of Na_2SO_4 0,5 M for 20 minutes. The cracking cell which results is then pelletized and the pellet part is soluted with millique water and then both analyzed using electrophoresis polyacrilamid-solium dodecyl sulfate or SDS-PAGE (Dwyer, et al., 2014). SDS-PAGE analysis is done using 20% tris-glysin.

III. RESULTS AND DISCUSSION

A. MSC

The synthetic gen of 4x SUPEL is synthesized in the GenART company, in the United States.

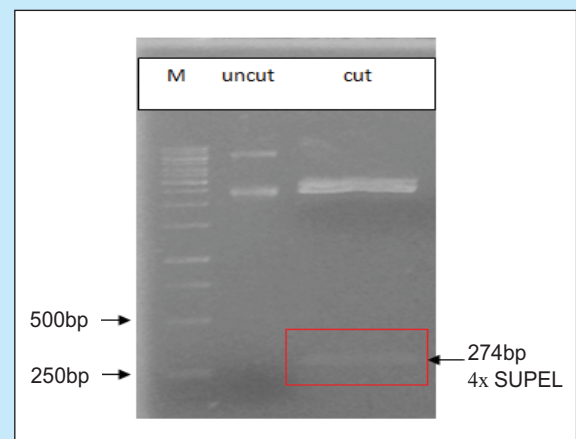


Figure 1
Electrophoregram of 4 threads SUPEL output from cloning vector.

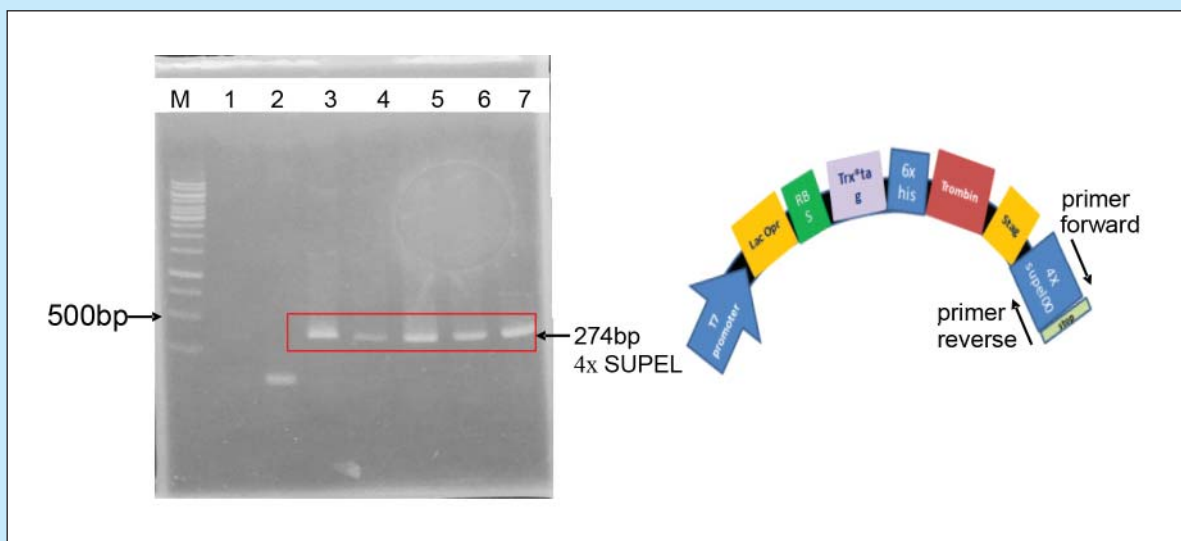


Figure 2
Confirmation result of 4 threads SUPEL synthetic gen using PCR
(M: Marker, 1; Control, Number 2 - 7: 4x SUPEL Plasmid Clon).

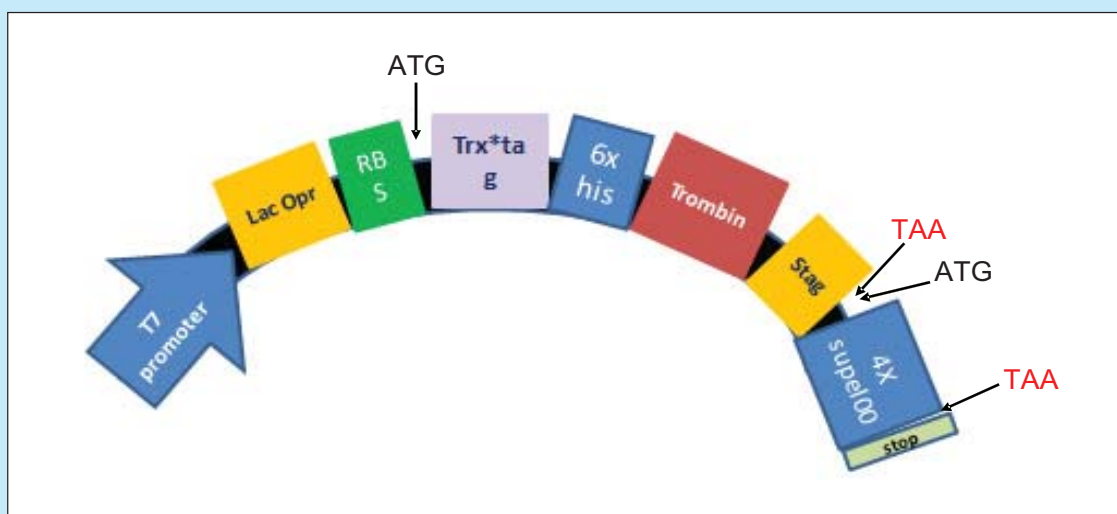


Figure 3
Location map where 4x SUPEL located in pET 32b expression vector.

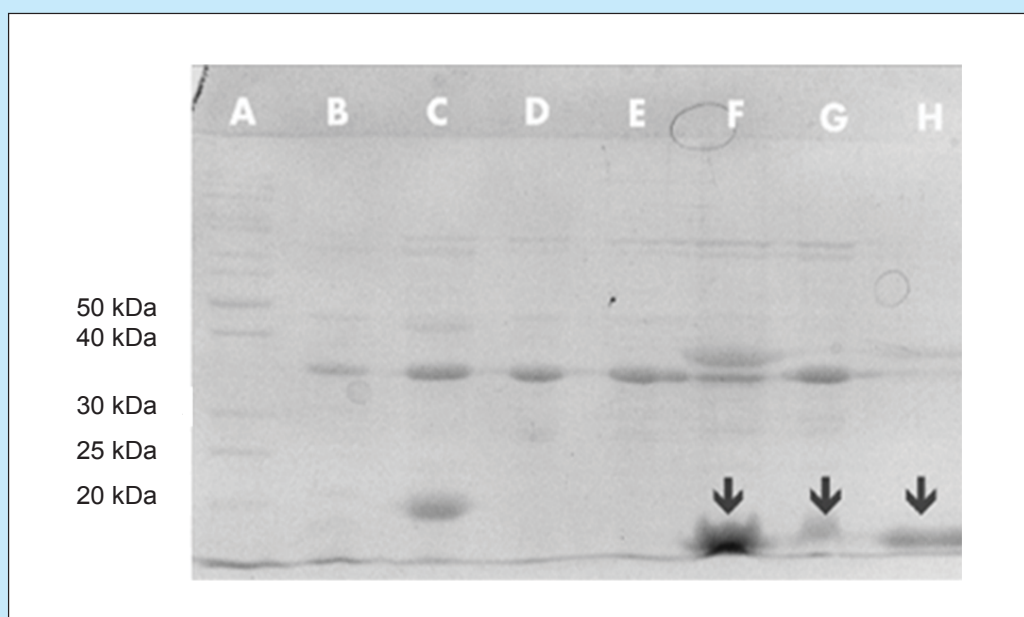


Figure 4
SDS-PAGE of 4x SUPEL protein A: Ladder, B: BL 21 non-pET (induction), C: pET wild type (induction), D: pET wild type (non-induction), E: MSC (non-induction), F: MSC (induction, heat at 90°C, and added with Na₂SO₄).

That gen is designed to have BamHI and HindIII restrictions so 4 threads SUPEL can be inserted in a multi cloning site in a pET 32b expression vector. Figure 1 shows the output of 4 threads SUPEL from the cloning vector using the restriction vector. The size of 4 threads SUPEL is 270 bp. This cutting product is then cloned into a pET 32b expression

vector which has been cut with the same enzyme. Beside using a restriction enzyme, pET recombinant that results in 4x SUPEL is confirmed with the PCR method (Figure 2).

The result of PCR confirmation shows that there is one thick band with a size of around 300 bp at clon number 3 - 7, whereas clon number 2 does not bring

inserted 4x SUPEL. Sample number 1 is negative control (no plasmid) using water as PCR template (Figure 2). Based on sequencing result of SUPEL 4x, known that the size of 4x SUPEL is in range of 270 bp. The sequence location in pET 32b expression vector is shown at Figure 3.

B. Analysis of Expression 4x SUPEL Recombinant

The protein of 4x SUPEL is not fused with other protein in a pET 32b expression vector. MSC construction is in regulation control of T7 promoter and to express target gen needs induction of IPTG 0,1 mM. The expression position of 4x SUPEL protein is a in size above 10 kDa (Figure 4). This condition is suitable with a prediction analysis result of molecular weight in insilicoi, 9,71 kDa.

Analysis of SDS-PAGE describes that 4x SUPEL protein can be expressed at a condition induced by IPTG 0,1 mM; induction and heat it up to 90°C; and also induction, heat at 90°C and with the addition of Na₂SO₄. This optimization result shows that 4 threads SUPEL peptide can be harvested a high temperature condition. This condition is very profitable for the oil industry if this 4x SUPEL is then proven as a surfactant. Analysis of the results of SDS-PAGE indicates that 4x SUPEL construction has been successfully done and the protein has been successfully expressed. Prediction of 4x SUPEL protein structure modelling is analyzed using <http://swissmodel.expasy.org/> (Figure 5). Based on a prediction of 4x SUPEL structure modelling at normal temperature, the structure is seen to have folded up. This condition shows that at normal temperature, a peptide will fold up so the surfactant characteristic is alleged to be not detected. Based on the optimization result with cell cracking at 90°C, 4x SUPEL is still also able to produce 4 threads of peptide with the suitable size. This condition determines that extraction process can be so simple and needs only heating to a temperature of 90°C.

The sequence of amino acid in SUPEL causes a peptide that two different side chain polars, hydrophobic and hydrophilic. Around 35% of amino acid is hydrophobic and 65% is hydrophilic. Modelling result at 4x SUPEL construction is expected to the produced 4x SUPEL can strengthen the act possibility as surfactant. 4 threads peptide surfactant has been designed using amino acid methionim (M) and aspartate acid (D) which both are in the group of hydrophilic's amino acid. Even so, the tail of SUPEL peptide is also designed to have

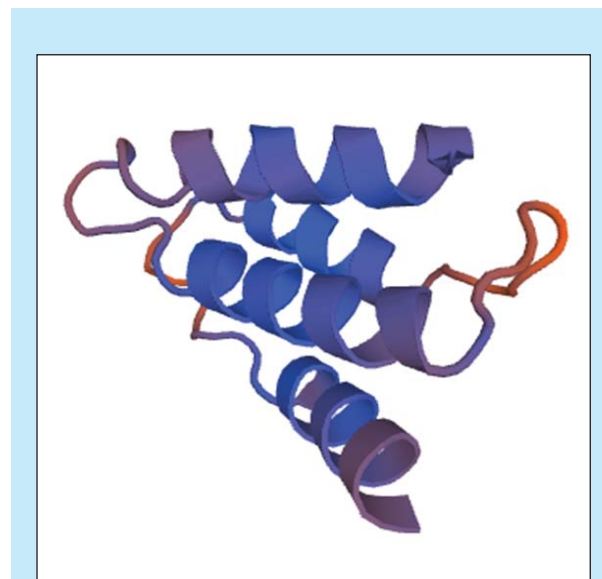


Figure 5
The 4x peptide form 4x alpha-helix
shown by blue color
(software online <http://swissmodel.expasy.org>).

three residual hydrophobic amino acids, arginin (R), lysin(K) and asparagin (N). This design has been successfully made by Middleberg and Dwyer, 2011 and also Middleberg et al. 2008 with different peptide sequence with SUPEL. Therefore, this further characteristic test for 4 threads SUPEL construction must be done in the order to know the function as a surfactant. This research shows 4 threads SUPEL peptide has been successfully produced with recombinant system only extracted at 90°C and it is to be expected that the detected surfactant activity is only at above normal temperature, where secondary structure can be opened.

IV. CONCLUSIONS

The construction of 4x SUPEL (MSC) has been successfully done into a pET 32b expression vector. The MSC construction is in regulation control of T7 promoter and to express target gen needs an induction of IPTG 0,1 mM. The expression position of 4x SUPEL protein is in a size larger than 10 kDa. This condition is suitable with a prediction analysis result of molecular weight in insilicio, 9,71 kDa. This optimization result determines that 4 threads SUPEL peptide can be harvested at a high temperature. If this 4x SUPEL is proven to have a surfactant characteristic through the surfactant characterization test, then this product will have enormous potential to be used for surfactant EOR.

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